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Exhibit A

MEMORANDUM

DATE: March 13, 1989

TO: Tom Boone & Keith Langley

FROM: Patricia Fausset and Hsieng Lu *PL*RE: N-terminal Sequence Analysis of Recombinant Human Collagenase Inhibitor (HCI) (Sample #020389B)

1. HPLC of rHCI:

One sample of HCI (0.5mg/ml) was submitted to this laboratory for N-terminal sequence analysis. Appr. 10ug of the sample was loaded directly onto a Protein C4 column and run on RP-HPLC. The chromatogram generated is shown below:



The result indicates that the sample chromatographed as a single, symmetrical peak at retention time of 78.8min. which elutes at appr. 41%B mobile phase (in 90% CH₃CN and 0.1% TFA).

2. N-terminal sequence analysis:

Appr. 500 pmol of the sample was loaded onto a sequencer and the first 18 amino acids were sequenced. Two sequences were detected:

1. Major Sequence (86%): (C)-S-(C)-S-P-V-H-P-Q-Q-A-F-(C)-N-A-D-V-V...
2. Minor Sequence (14%): S-(C)-S-P-V-H-(X)-Q-(Q)-A-F-(C)-N-(X)-D-V...

[where(X)=amino acid not positively assigned from sequencing]

The minor sequence indicates that the first cysteine at the N-terminus has been processed. No methionyl sequence is detected in this sample.

3. C-terminal Analysis:

The protein was digested with carboxypeptidase P at 25C and aliquots were taken at different time points (5, 10, 20, 40, 60, and 120 min.) and analyzed for amino acids released by the digestion. However, carboxypeptidase P poorly digested HCl. Many amino acids were released at poor rates and cannot be correlated well with the expected C-terminal sequence. Thus, the precise C-terminal sequence cannot be assigned from the experiment. However, all the C-terminal amino acids up to 14 residues from the C-terminal proline are present in the digest. To further confirm the intactness of the C-terminus for HCl, we suggest that isolation of C-terminal tryptic peptides should be performed.

cc: B. Altrock, L. Souza, D. Vapnek